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Preparation and characterization of paclitaxel nanosuspension using novel emulsification method by combining high speed homogenizer and high pressure homogenization

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ABSTRACT

The aim of this study was to develop an alternative, more bio-available, better tolerated paclitaxel nanosuspension (PTXNS) for intravenous injection in comparison with commercially available Taxol® formulation. In this study, PTXNS was prepared by emulsification method through combination of high speed homogenizer and high pressure homogenization, followed by lyophilization process for intravenous administration. The main production parameters including volume ratio of organic phase in water and organic phase (V_o:V_{w+o}), concentration of PTX, content of PTX and emulsification time (E_t), homogenization pressure (HP) and passes (P_s) for high pressure homogenization were optimized and their effects on mean particle size (MPS) and particle size distribution (PSD) of PTXNS were investigated. The characteristics of PTXNS, such as, surface morphology, physical status of paclitaxel (PTX) in PTXNS, redispersibility of PTXNS in purified water, *in vitro* dissolution study and bioavailability *in vivo* were all investigated. The PTXNS obtained under optimum conditions had an MPS of 186.8 nm and a zeta potential (ZP) of −6.87 mV. The PTX content in PTXNS was approximately 3.42%. Moreover, the residual amount of chloroform was lower than the International Conference on Harmonization limit (60 ppm) for solvents. The dissolution study indicated PTXNS had merits including effect to fast at the side of raw PTX and sustained-dissolution character compared with Taxol® formulation. Moreover, the bioavailability of PTXNS increased 14.38 and 3.51 times respectively compared with raw PTX and Taxol® formulation.

1. Introduction

Paclitaxel (PTX), as a kind of anticancer drug isolated from the bark of *Taxus brevifolia*, has demonstrated high and wide antitumor activity (Xu et al., 2005; Zhao et al., 2010). However, PTX was administrated as Taxol® formulation added Cremophor EL as an adjuvant due to its low solubility. Unfortunately, Cremophor EL induces hypersensitivity and causes serious side-effects reactions in many patients (Michaud et al., 2000; Szebeni et al., 1998). Attempts to improve therapeutic effects of PTX and reduce its side effects through solubilizing poorly water-soluble PTX using various delivery systems, such as micellar carriers (Kan et al., 1999; Tarr et al., 1987; Zhang et al., 1996), soluble polymers (Cristina et al., 2002; Jong-Ho et al., 2006), liposome formulations (Dong et al., 2009; Lee et al., 2007) have been of limited success. Simultaneously, albumin-bound formulation of PTX (Abraxane® ABI-007) came into being and achieved great success on the poor solubility of PTX. However, the price of ABI-007 is very expensive for some patients because of adding human serum albumin. Thus, it is necessary to find a new formulation with advantages of high water solubility and low cost.

An alternative formulation is nanosuspension which is composed of submicron drug particles (amorphous or crystalline) suspended in a dispersion medium (mostly water) and stabilized by polymer(s) or surfactant(s) (Rabinow, 2004). The small particle size and high surface area to volume ratio make nanosuspension increase saturation solubility and dissolution rate of drug particles (Patravale et al., 2004). Besides, nanosuspension has the

Abbreviations: DLS, dynamic light scattering; DSC, differential scanning calorimetry; DSPE-mPEG, 1, 2-distearoyl-phosphatidyl ethanolamine-methyl-polyethyleneglycol; E_t, emulsification time; GC, gas chromatograph; HP, homogenization pressure; HPH, high pressure homogenization; HPLC, high performance liquid chromatography; IgG, intravenous immuno globulin; MPS, mean particle size; NMP, *N*-methylpyrrolidone; PSD, particle size distribution; P_s, passes; PTX, paclitaxel; PTXNS, paclitaxel nanosuspension; SEM, scanning electron microscope; TGA, thermogravimetric analyzer; V_o:V_{w+o}, volume ratio of organic phase in water and organic phase; XRD, X-ray diffraction; ZP, zeta potential.

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advantages of stability enhancement (Moschwitzer et al., 2004; Muller and Keck, 2004), variety of therapeutic applications (Sun et al., 2011), and passive targeting effects (Peters et al., 2000).

Particle size reduction to nanometer range can be achieved through precipitation (Trotta et al., 2001), media milling (Merisko-Liversidge et al., 1996) and high pressure homogenization (HPH) (Xiong et al., 2008). The HPH technique has several advantages over the others, for example, simplicity of the process, cost saving, ease of large scale production and reduced product contamination.

PTX-loaded nanoparticles have showed high water solubility and a variety of advantages compared with standard commercial formulations of PTX (Desai et al., 2006; Gradishar et al., 2005). It has been reported that polymers have been used to develop PTX-loaded nanoparticles including bovine serum albumin (BSA) (Zhao et al., 2010), poly(lactic-co-glycolic acid) (PLGA) (Fonseca et al., 2002), chitosan (Jong-Ho et al., 2006), polyethylene glycol (Zhiping and Si-Shen, 2006). However, water soluble micromolecule as carriers loading PTX has not been prepared into nanosuspensions. Mannitol is a sugar alcohol and commonly used in pharmaceutical freeze-dried formulations to improve their physical and chemical stability (Abdelwahed et al., 2006). Mannitol is extensive and cheap in contrast with polymers mentioned above. Moreover, serving as carrier and cryoprotectant in our study supplied it with both time-saving and economical potential.

It has been reported that Lee et al. (2013) and Wang et al. (2011) used HPH to prepare PTX nanosuspension (PTXNS). However, the preparation methods in the two literatures were found to be equipped with the following disadvantages. As for Lee's literature, the drawbacks consist of incomplete removal of NMP, time-consuming and cost-consuming caused by the reuse of HPH and surfactant. Because NMP is a kind of organic solvent with high chemical stability and thermal stability, it was hard to eliminate NMP almost completely only relying on centrifugation in view of the solubility of NMP in water. When it comes to the preparation technology of PTXNS in Wang's study, it is apparent to learn that high usage of surfactants including Poloxamer 188 and PEG-400 will bring about large side-effects and high expenses. At the same time, high pressure, large number of passes (P_s) and reuse of HPH will lead to waste of manpower, money and time.

To overcome the drawbacks of preparation methods of PTXNS above-mentioned, a novel and new strategy was put forward in our present study, namely the combination of two emulsification methods. First, high speed homogenizer was employed to form crude PTX o/w emulsion. In this emulsion chloroform and ethanol was chosen to be oil phase. Meanwhile, deionized water saturated by chloroform along with mannitol as excipient and poloxamer 188 as surfactant was chosen to be water phase. Next, HPH technique was utilized to treat the crude emulsion to fulfill further nanosized emulsification. The residual organic solvent was eliminated through vacuum rotary evaporation at 40 °C. The process of preparing PTXNS was optimized through experiments designed to determine the best operational conditions. The novelty of the production strategy is based on the following respects. Deionized water being saturated by chloroform was chosen to be water phase to improve the stability of emulsion relying on blocking the spread of the drug solution to water. So drug precipitation was reduced. Chloroform and ethanol were easily removable to promote drug safety due to their low boiling point. Low usage of surfactant, namely poloxamer 188 would bring about smaller side-effects and lower expenses. Moreover, HPH was just used for once and P_s were no more than 7, which saved lots of manpower, money and time.

In the present study, characterizations of the nanosuspension, including the PTX content in PTXNS freeze-dried powder, mean particle size (MPS) and zeta potential (ZP) were carried out. In addition, surface morphology and physical status of PTX in PTXNS were also investigated. The redispersibility of dry powder in purified water through lyophilization process was tested. The dissolution characters and bioavailability of PTXNS were also researched in the present work.

2. Materials and methods

2.1. Materials

The PTX with a purity of mass fraction of more than 98.5% was kindly provided by Hisun Pharmaceutical Co., Ltd. (Zhejiang, China). Phosphate-buffered saline (pH 7.4), mannitol, poloxamer 188 were all obtained from Sigma–Aldrich (St. Louis, MO, USA). Acetonitrile and methanol were of high performance liquid chromatography grade. Chloroform (purity > 99.5%), ethanol (purity > 99.5%) and the other reagents were all analytical grade.

2.2. Preparation of PTXNS

A flow chart of the preparation processes was shown in Fig. 1. PTX was dissolved in chloroform as the solvent and ethanol as the co-solvent (chloroform:ethanol, 11:1, v:v), and dispersed 5–10 min in an ultrasonication bath TI-H-5 (Elma, Singen, Germany). In the following emulsification process, chloroform and ethanol were the organic phase of emulsion. The solution was added dropwise slowly to deionized water being saturated by chloroform along with mannitol as excipient and poloxamer 188 as surfactant that

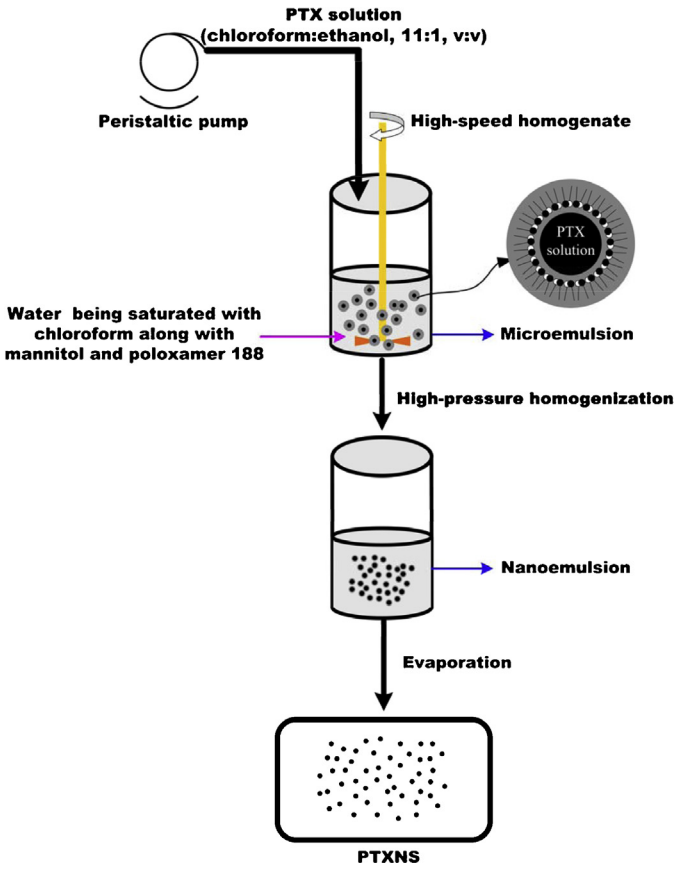

Fig. 1. The flow chart of the experimental processes.